

Amendments to the Claims

Claim 1 (Currently amended): A method of assaying for protease activity inside a cell, comprising:
providing introducing into a cell a nucleic acid construct having a sequence encoding an amino terminal portion of a fluorescent reporter fused to a sequence encoding a substrate of a protease followed by a sequence encoding a carboxy-carboxyl terminal portion of a-the fluorescent reporter protein;
expressing a-the recombinant fluorescent substrate in the presence of the-a protease;
detecting a change in quenching of fluorescence in the recombinant fluorescent substrate as an indication of protease activity.

Claim 2 (Original): The method of claim 1 wherein the presence of a peptide bond between the amino and carboxyl-terminal fragment of the fluorescent substrate is essential to generate or maintain fluorescence.

Claim 3 (Original): The method of claim 1 wherein fluorescence is quenched by cleavage in the protease substrate sequence.

Claim 4 (Original): The method of claim 1 wherein the intrinsically fluorescent protein is GFP.

Claim 5 (Currently amended): The method of claim 1 wherein the protease is introduced into a cell by expression from a nucleic acid construct.

Claim 6 (Currently amended): A method for identifying a protease that cleaves a target amino acid sequence inside cells, comprising:
providing introducing into a cell a nucleic acid construct having a sequence encoding an amino terminal portion of a fluorescent reporter fused to a plurality of sequences each encoding a desired substrate target followed by a sequence encoding a carboxy-carboxyl terminal portion of a-the fluorescent reporter protein;

expressing of the recombinant fluorescent substrate in the presence of a the plurality of proteases; detecting at least one of the plurality of proteases that recognize the target sequence by quenching of the fluorescence of the reporter.

Claim 7 (Original): The method of claim 6 wherein the fluorescent reporter protein is GFP.

Claims 8-13 (Cancelled)

Claim 14 (New): A method of assaying for protease activity inside a cell, comprising: introducing into a cell a nucleic acid construct having a sequence encoding an amino terminal portion of a green fluorescent reporter protein fused to protease followed by a sequence encoding a carboxyl terminal portion of the green fluorescent reporter protein; expressing the recombinant fluorescent substrate in the presence of a protease; detecting a change in quenching of fluorescence in said substrate as an indication of protease activity.

Claim 15 (New): The method of claim 14 wherein the presence of a peptide bond between the amino and carboxyl-terminal fragment of the serine substrate is essential to generate or maintain fluorescence.

Claim 16 (New): The method of claim 14 wherein fluorescence is quenched by cleavage in a serine protease substrate sequence.

Claim 17 (New): The method of claim 14 wherein the protease sequence is a serine protease or a mutant thereof.

Claim 18 (New): The method of claim 17 wherein the serine protease is NS3/4A.

Claim 19 (New): The method of claim 17 wherein a mutant serine protease has a serine converted to a glycine.

Claim 20 (New): A method of assaying for protease activity inside a cell, comprising:
introducing into a cell a nucleic acid construct having a sequence encoding an amino terminal portion of a green fluorescent reporter protein fused to a serine protease sequence that encodes a serine protease followed by a sequence encoding a carboxyl terminal portion of the green fluorescent reporter protein;
expressing the serine substrate in the presence of a protease;
detecting a change in quenching of fluorescence in said substrate as an indication of protease activity.

Claim 21 (New): The method of claim 20 wherein the presence of a peptide bond between the amino and carboxyl-terminal fragment of the serine substrate is essential to generate or maintain fluorescence.

Claim 22 (New): The method of claim 20 wherein fluorescence is quenched by cleavage in a serine protease substrate sequence or a mutant thereof.

Claim 23 (New): The method of claim 22 wherein the serine protease sequence is NS3/4A.

Claim 24 (New): The method of claim 22 wherein the mutant has a serine converted to a glycine.

Claim 25 (New): A method of assaying for protease activity inside a cell, comprising:
introducing into a cell a nucleic acid construct having a sequence encoding an amino terminal portion of a green fluorescent reporter protein fused to a serine protease sequence that encodes a serine protease followed by a sequence encoding a carboxyl terminal portion of the green fluorescent reporter protein;
expressing the serine substrate in the presence of a protease;
purifying the serine protease substrate;
detecting a change in quenching of fluorescence in said substrate as an indication of protease activity.

Claim 26 (New): The method of claim 25 wherein the presence of a peptide bond between the amino and carboxyl-terminal fragment of the serine substrate is essential to generate or maintain fluorescence.

Claim 27 (New): The method of claim 25 wherein fluorescence is quenched by cleavage in a serine protease substrate sequence or a mutant thereof.

Claim 28 (New): The method of claim 27 wherein the serine protease sequence is NS3/4A.

Claim 29 (New): The method of claim 27 wherein the mutant has a serine converted to a glycine.